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AT OPTIMUM TEMPERATURES AND THE CYTOLOGY
OF THEIR ACTIVE STAGES

FRANK B. COTNER

A DISSERTATION
SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
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INTRODUCTION

Any satisfactory study of the cytology of the zoöspores of the Oömycetes requires a sound understanding of what constitutes healthy conditions for their development and activity. Hitherto no means of obtaining perfectly healthy zoöspores in large numbers under optimal conditions had been perfected. Since the optimal conditions of development were determined for each species by a rigorously controlled experimental procedure, the study has two chief aspects: the physiological, dealing with the conditions determining zoöspore production under optimum and other conditions; and the cytological, dealing primarily with the mechanism of cilia formation.

The morphology and especially the ciliation of zoöspores has been of interest to botanists since the earliest microscopic studies of the thallophytes. Several phylogenetic classifications have been constructed around the types of ciliation found in zoöspores of different groups of the fresh water algae and reference has been frequently made in mycological literature to the details of zoöspore formation and ciliation in fungi. In spite of the biological importance of zoöspores and the long-continued interest in them, comparatively little is known concerning significant details in the formation of the cilia, either in the algae or the fungi. With the exception of the investigations of Klebs (27) and Kauffman (25, 26) relatively little is known in genera other than *Saprolegnia* regarding the stimuli which bring about the formation of sporangia and zoöspores and the conditions most favorable for the maximum activity of the latter.

HISTORICAL

In many cases the cilia emerge from a specially differentiated part of the protoplast; the literature dealing with this question is reviewed by the

¹ Paper from the Department of Botany of the University of Michigan no. 321.

writer (12) in another paper. It is evident that in a number of genera of algae and fungi, the insertions of the cilia are in chromatic basal granules just inside the plasma membrane. These basal granules have been identified as blepharoplasts. West and Fritsch (44) have said, however, that the general occurrence of blepharoplasts in motile protophyta is still in doubt.

There have been numerous references in the literature to the temperature factor in so far as it favors zoospore formation in various genera of the Oömycetes. De Bary (3) first reported that in *Cystopus* zoospore formation may occur at any temperature between 5° and 25° C. Klebs (27) has shown that in *Saprolegnia mixta* de Bary, at 1° to 2° C., zoöspores are liberated after 48 hours; at 6° to 8° C., zoöspores are liberated after 24 hours, while the optimum lies between 24° and 28° C. At the latter temperature zoöspores are formed in from 5 to 6 hours while the maximum temperature for this process lies between 32° and 33° C. Melhus (30) has shown that zoöspores may be formed by the conidia of *Cystopus candidus* (Pers.) Lév. at temperatures ranging from very near zero to 25° C., and that the optimum is 10° C. A number of investigators have mentioned the temperature-relation for zoospore formation in the genus *Phytophthora*. Melhus (31) points out that there is a very definite temperature-relation for zoospore formation in *Phytophthora infestans* (Mont.) de Bary and that the optimum temperature lies between 12° and 13° C. Rosenbaum (38) who worked with various species of *Phytophthora*, states in his discussion of zoospore formation by the conidia, that "the suspension of spores was . . . held at a temperature of about 15° C." and further that "at the end of from two to five hours a large number of the conidia had germinated." Coker (9) at various places in his discussion of the different species of the Saprolegniaceae, has merely mentioned the temperatures at which certain members of the family form zoöspores. In *Saprolegnia anisospora* de Bary, the spores are said by Coker to be liberated in spring water at a temperature of 13° C. The temperature at which zoöspores were found to be formed in other genera and species discussed by Coker were as follows: *Isoachlya toruloides* Kauff. & Coker, 26° C.; *I. unispora* Coker & Couch, 30° C.; *Protoachlya paradoxa* Coker, 26° C.; *Achlya apiculata* de Bary, 22° C.; *A. klebsiana* Pieters, 18–24° C.; *A. orion* Coker & Couch, 21.5° C. In his discussion of *Apodachlya brachynema* (Hildeb.) Prings. he says "The sporangia are very whimsical about opening at room temperature (about 60–70° F.) but rarely fail to discharge their spores if kept in an ice box." Jones and Drechsler (24), working with *Aphanomyces euteiches* Drechsler, reported that "with young thalli at a temperature of about 20° C., evacuation of the sporangial filaments was found to begin about six to seven hours after washing was completed," and have shown that the temperature range for spore formation in this fungus is from 9° to 11° C. to 33° to 35° C. Nishimura (34) has reviewed the literature regarding zoospore formation in the genus *Plasmopara* and great divergence is shown to exist in the data

given by investigators who have studied temperature relations to zoöspore formation in these plants.

MATERIAL AND METHODS

In the writer's work twelve Oömycetes were used: *Allomyces arbuscula* Butler, contributed by Dr. Bessie B. Kanouse of the University of Michigan Herbarium, who obtained it from Dr. W. C. Coker of Chapel Hill, North Carolina, March 6, 1926, where it was isolated from soil under grass; *Apodachlya brachynema* (Hildeb.) Prings., contributed by Dr. Kanouse, who collected it on submerged fruits of apple and of *Crataegus*, Ann Arbor, Michigan; *Rhipidium europaeum* von Minden, collected by Dr. Kanouse on submerged fruit bait exposed in a spring fed pond, March 20, 1929, Ann Arbor, Michigan; *Saprolegnia monoica* var. *glomerata* Tisenhausen, contributed and determined by Dr. Kanouse from a culture collected near Ann Arbor in 1925; *Isoachlya paradoxa* (Coker) Kauffman, was contributed by Dr. Kanouse. It was found near Ann Arbor June 10, 1926, identified by Dr. Kauffman; *Achlya conspicua* Coker, contributed by Dr. Kanouse, collected on a submerged apple in a muddy ditch, March 20, 1929, Ann Arbor, Michigan; *Aphanomyces euteiches* Drechsler, received by Dr. Kanouse² from Dr. C. Drechsler of Washington, D. C.; *Phytophthora cactorum* (Cohn & Lebert) Schroeter, obtained from Professor H. H. Whetzel of Cornell University, isolated from apple August 25, 1920; *P. erythroseptica* Pethybridge, obtained from Professor H. H. Whetzel, October 31, 1920; *P. palmivora* Butler, obtained from Dr. L. H. Leonian, of Morgantown, West Virginia, who obtained it from Butler by way of Pethybridge. Leonian (29) states that the organism was isolated by Ashby from Cacao fruit in the West Indies; *P. terrestris* Sherbakoff, sent by Dr. D. Reddick, November 4, 1920, who obtained it from Dr. Sherbakoff; *Phytophthora* sp., obtained from Dr. D. Reddick of Cornell University, November 4, 1920. This plant was isolated from tomato by Dr. Reddick.

All cultures used were grown from single-spore cultures isolated by the method developed by Kauffman (25). With the exception of cultures of the genera *Phytophthora* and *Allomyces*, they were grown on sterile pea broth for 24 to 48 hours. Young vigorous cultures were used in all cases.

Subcultures were made by using sterile pea broth and adding a very small portion of mycelium taken from a similar culture not more than four days old. So-called conductivity water³ was used in all cultures. The mycelium actually used in the experiments was taken from a vigorous culture which was 24 or 48 hours old which had been grown either at room

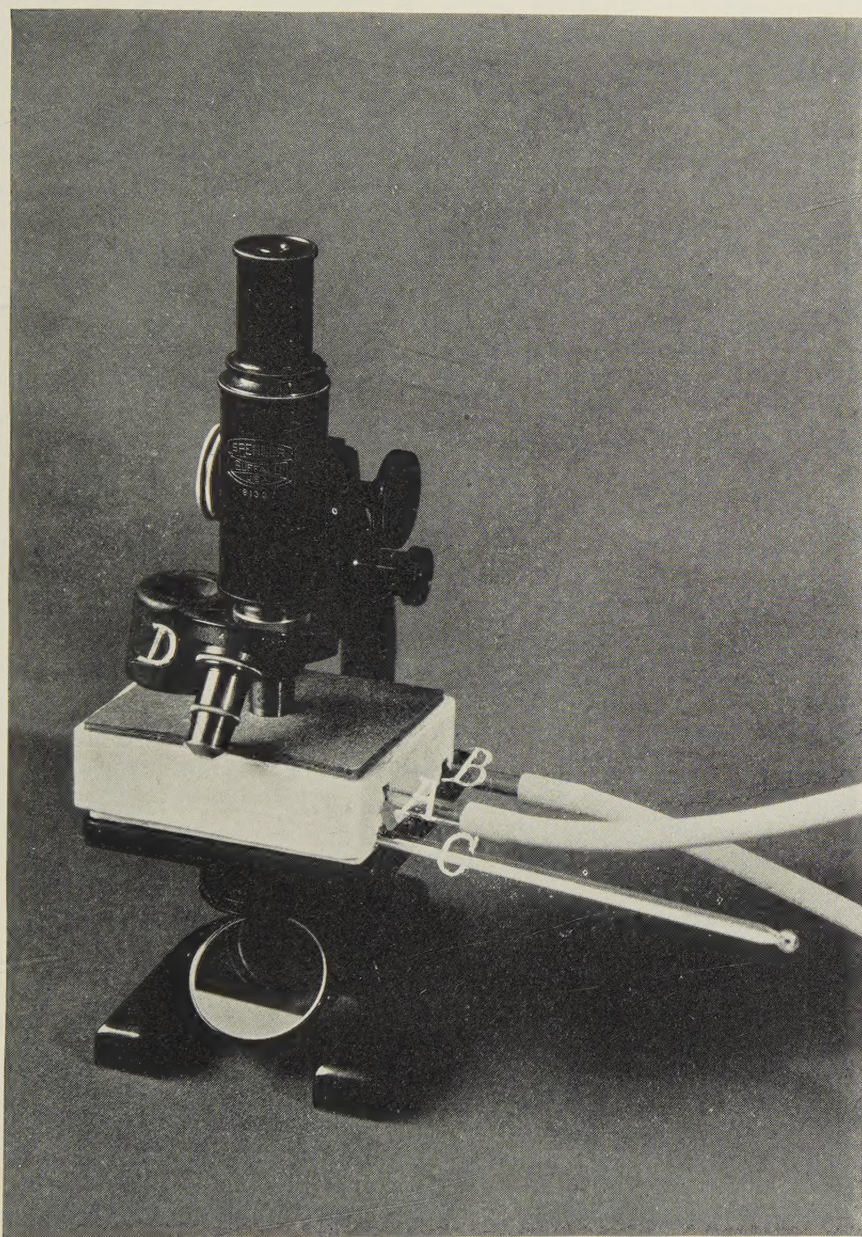
² For providing the many cultures and for numerous helpful suggestions thankful acknowledgment is hereby made by the writer to Dr. Kanouse.

³ The best results were obtained by the use of water free from the minute trace of metallic ions which occurs in most ordinary distilled water. It was possible to obtain from the chemical laboratory water redistilled in quartz, used for electrical conductivity measurements.

temperature (usually between 20° and 22° C.), or in a cold room at a temperature varying between 10° and 12° C. It was necessary to allow the cultures in the cold to grow at least one additional day before sufficient mycelium had developed. It was found that for the major part of the experiments the cultures in stock pea broth which had been grown at temperatures varying between 20° and 22° C. were the most satisfactory, since the most uniform results were obtained from mycelium which had grown rapidly and was in a vigorous condition.

Cultures of *Allomyces arbuscula* were grown on sterile flies in sterile conductivity water at 26° C. until a good development of mycelium was obtained. The mycelium was used before there was any indication of developing sporangia or oögonia. The mycelium from the young culture in pea broth, in the case of species of *Achlya*, *Aphanomyces*, *Isoachlya*, and *Saprolegnia*, and from the young culture on the fly in the case of *Allomyces arbuscula*, was removed either by cutting out a portion with sterile scissors or by tearing out one piece with a sterile platinum hook. This young vigorous mycelium was washed for one hour through six changes of sterile conductivity water, then mounted in hanging drops of well-aërated sterile conductivity water. The preparations were kept in *micro-stage constant temperature chambers* which were placed on the stages of a row of microscopes each under a 16-mm. objective for continued observation during the considerable time required for the development of the sporangia and the liberation of the zoöspores. When a comparable series of experiments was being run in these chambers to determine optimum temperatures, lights for all of the microscopes in use were turned on and off at the same time in order to have all conditions excepting temperature exactly alike for the entire series.

In the experiments dealing with the effect of temperature on the development of sporangia and the formation of zoöspores, warm and cold micro-stages were used for constant temperature chambers. The warm stage used was the micro-slide incubator for electric heating, manufactured by the Chicago Surgical Equipment Company. This instrument is adjustable for temperatures from room temperature to slightly above 40° C. and permits of exact control for all of the higher temperatures of the experiments. For the work below room temperatures a cold stage was used (text fig. 1) which was constructed by the writer, by using 8-mm. glass tubing bent into a rectangular double coil allowing just sufficient space inside the coil for the easy placing and removal of a standard microscope-slide. A coil with two complete turns was found to have adequate cooling surface when properly insulated from without. The insulation was accomplished by gluing together several thicknesses of cardboard in the form of a rectangular box and cementing a glass window in the bottom over which the slide containing the hanging drop mount could be placed. The chamber was covered by means of two thicknesses of paper felt such as is used in drying plants for the herbarium. A hole was made in the center of these



TEXT FIG. 1. Low temperature chamber mounted on stage of a microscope ready for use, with intake at *A*, drain at *B*, thermometer at *C*, and weight *D*.

insulators just large enough to permit the insertion of the objective of the microscope and thus enable one to observe the mount without opening the constant temperature chamber. A thermometer was mounted with the bulb near the position of the slide in the chamber and with the part containing the scale protruding outside the insulating walls, thus enabling one to take temperature readings at will. The two ends of the coil were also allowed to protrude six inches or more outside the insulation. One end was connected to a cold water tap by means of rubber tubing, and the other allowed to drain into a sink. Text figure 1 shows this low temperature chamber mounted on a microscope stage with intake at *A*, drain at *B*, and thermometer at *C*. The weight *D* holds the chamber firmly on the stage of the microscope but permits movement of the apparatus; this enables one to orient the mount for observation under the microscope without affecting the temperature of the mount. With the temperature of the tap water varying around 13° C. it was possible easily to control the temperature of the chamber to as low as 17° C. by regulating the rate of flow of cold water through the coils. When temperatures slightly below 17° C. were desired, this could be accomplished by introducing a coil which was immersed in an ice bath between the water tap and the constant low temperature chamber. For the experiments in which it was necessary to have very low temperatures, as for example from 5° to 14° C., the most satisfactory results were obtained by working in a cold room with the temperature at 5° C. or lower. The temperatures above that of the cold room in which the experiment was set up were obtained by the use of the low temperature chamber; the water circulating through the coil in this case serves to raise the temperature to the desired degree. It was found most satisfactory to work in the cold room for all temperatures below 17° C. and raise the temperature of the low temperature chamber by circulating warm water through the coil.

For the study of the second swimming stage of the diplanetic species the most satisfactory and uniform results were obtained by removing the mycelium with the attached sporangia and replacing the water in which the spores of the first swimming stage had been liberated. This was accomplished by removing the cover glass and the hanging drop containing the material from the hollow ground slide. The mycelium and sporangia were then carefully removed after which the drop of water was drained away by means of a very fine capillary pipette. Since many of the spores come to rest on the surface of the drop one must exercise great care in order to avoid removal of the encysted spores. Well aerated fresh conductivity water was then added and the preparation returned to the slide and replaced under favorable conditions for further study.

The technique of killing and staining the zoöspores has been given by the writer in a previous paper (12). In the following work the technique used is essentially the same as that described for the zoöspores of *Blasto-*

cladia. In view of the fact that there has been some improvement in the staining technique since writing that paper, it may not be out of place to give a brief outline of the methods used in this work.

After the hanging drop containing the zoöspores had been exposed to the fumes of 1 percent osmic acid for from 30 to 60 seconds, an equal volume of a .005 percent aqueous crystal violet was added to the drop of spore suspension on a number one cover glass. The exposure to the osmic acid fumes and the concentration of the staining solution were varied occasionally in order to bring out the details of the structure of the different zoöspores. The preparation was then dried in a desiccator over sulfuric acid for from 24 to 36 hours. Spores which tend to take the stain less readily than others can be stained in a highly satisfactory manner by allowing the preparation to dry more slowly after the addition of the dilute staining solution. This allows more time for the stain to diffuse into the protoplasm of the spores. This was accomplished by simply allowing the preparation to air-dry either partially or completely. The final dehydration of the preparation was in all cases finished in the desiccator for all of the work carried on in the moist atmosphere at Ann Arbor, but in the dry high altitude of Bozeman, Montana, where part of the work was done, air-drying was sufficient.

In order to prevent the reabsorption of moisture, clove oil was added to the stained area on the cover glass as soon as the desiccator was opened and before removal of the preparation. After extraction and clearing in clove oil for from a few minutes to one half hour, depending on the rate of extraction, the oil was removed with xylol and the preparation mounted on a slide in balsam.

All detailed studies and drawings were made with the aid of a 2-mm. apochromatic oil immersion objective, a 12 × compensating ocular and a camera lucida.

EXPERIMENTAL AND CYTOLOGICAL STUDIES

Blastocladales

In this order the zoöspores are uniciliate and monoplanetic. Of the three species of this order studied *Blastocladia globosa* Kanouse and *B. pringsheimii* Reinsch have been reported by the writer elsewhere (12). *Allomyces arbuscula* is discussed in this paper. The genus *Allomyces* as well as the species were described by Butler (7) in 1911 and the following year collected and studied in some detail by Barrett (2).

Temperature Relations.—This plant is more sensitive to temperature than many of the other members of this group of highly sensitive plants. The temperature range of zoöspore formation and liberation extends from 18° C. to 32° C. Sporangia are formed slightly above and below this range but they seem to be unable to complete the development and liberate the spores at these temperatures. Sporangia which are formed just outside

the temperature range of zoöspore formation, *e.g.*, at 33° C., when later subjected to a temperature at or near the optimum will readily form spores in a perfectly normal manner. The optimum temperature for zoöspore formation in these plants lies between 25° and 27° C. At this temperature, sporangia begin to be walled off one hour after the washing process is completed, and the first liberation of spores takes place in from two and one half to three hours after completion of the washing process. It will be noted that the optimum temperature for formation of sporangia and zoöspores is somewhat nearer the maximum than it is to the minimum temperature. The optima for sporangium formation and for zoöspore formation seem to be the same, whereas the range for sporangium formation is somewhat greater than for zoöspore formation.

Optimum conditions and normal development are defined in this paper in the same way as in a previous paper dealing with the zoöspores of *Blastocladia* (12). *Normal development may be defined as the development which takes place under optimum conditions. Optimum conditions are those which enable the greatest possible number of active and healthy spores to be formed and liberated in the briefest given time.* The spores which develop under optimum conditions are believed to exhibit the morphology and behavior typical for the species.

Development of the Sporangia and the Structure of the Zoöspores.—The developing sporangium is first observed as a slight swelling of the tip of the hypha; a slight constriction may be seen to develop a short distance from the tip indicating the position of the developing cross wall which forms as a ring from the periphery of the hypha toward the center. The walling off of the first sporangia under optimum conditions begins about one hour after completion of the washing process. The entire process of septum formation requires from one-half to three-fourths of an hour for completion. The maturation of the young sporangia takes place rapidly and under favorable conditions spores will begin to be liberated in from one and one-half to two hours after the first indication of developing cross walls. As described by Barrett (2), liberation of the first spores takes place into a vesicle, which persists until from four to six spores have emerged from the sporangium; the vesicle then ruptures, liberating the spores.

The zoöspores of *Allomyces arbuscula* have been described in some detail by Butler (7) and by Barrett (2). There are, however, a number of cytological details which need further consideration. Several striking similarities have been noted between the zoöspores of this plant and the uniciliate spores of the two species of *Blastocladia* studied by the writer (12). But there are also a number of significant differences; this necessitates the complete redescription of the zoöspores of this species.

The first spores that emerge from the sporangium are at first irregular in shape and often exhibit amoeboid changes as described by Butler. The immature cilium, if any has developed at this stage, is much shorter and

thicker than the cilium of the mature active spore. Plate XXX, figure 1, shows a spore with an immature cilium. It is quite evident that the development of the cilium is in some way intimately connected with one or several highly staining granules. There is always a main granule which occupies the position at the base of the cilium, and there are in addition often as many as three secondary granules which occupy positions at the base of threads connecting the basal granule and the nucleus (figs. 1 and 2). Many young spores show but one secondary granule and one thread connecting it with the basal granule. After the completion of the cilium, which seems to be accomplished by its being whipped about until it has attained sufficient length and delicacy to function as a propeller, these smaller granules, sometimes three in number, are drawn back toward the nucleus. In combination with several other chromatic granules they seem to form the tip of this organ (fig. 3).

There are also very definite connections between these secondary granules and the large central chromatic body in the nucleus. This body in turn is connected to the peripheral chromatic granules of the nucleus by delicate threads, so that the result is a rather coarse and meshed net-like structure (fig. 1). The peripheral chromatin is distributed in various-sized granules just inside the nuclear membrane. The nucleus is very similar in shape to the nuclei of the zoöspores of the species of *Blastocladia* studied; it is, however, usually somewhat smaller in the spores of *Allomyces arbuscula*. The rather large, very finely granular, cytoplasmic mass, lying just forward of the broader anterior part of the nucleus in which this broader part of the nucleus is imbedded, is considered by the writer to be homologous with the finely granular, dense mass of cytoplasm which has been described (12) for the spores of the genus *Blastocladia*. In favorable material of *Blastocladia* this forms a cap-like structure between the anterior end of the nucleus and the surface of the spore. This cap has been referred to as the food body by Barrett (l. c.) and is shown by him as a definitely outlined body limited by a membrane. Under certain conditions and particularly in the older spores this finely granular cytoplasmic body does appear to have a very definite exterior boundary line, while in younger material this boundary is often not well marked (figs. 1 and 3). This question will be discussed in more detail a little later in the paper. In the young spores the highly vacuolated cytoplasm of the extreme anterior end is often drawn out into a rather long-pointed tip. This condition may possibly be brought about in some cases by the young spore being forced out, ciliated end first, through the exit-pore of the sporangium, resulting in the crowding of this more pliable material toward the anterior end of the spore. In other cases this end may have been drawn out by becoming attached to the cilium of the spore immediately ahead of it. The latter explanation is probably more generally true, especially for those spores emerging later and after the development of the cilia. But whatever the

orientation of the young spores, this extreme anterior end is filled with highly vacuolated cytoplasm which contains many large granules that are either distributed through it (fig. 2) or are congregated about the denser cytoplasm (fig. 4). At favorable temperatures, after the spores have been allowed to swim for several hours, most of this highly vacuolated cytoplasm along with the large granules has moved back around the equatorial region of the spore; or it may even pass beyond this region toward the insertion of the cilium. In the young spores the equatorial region is not filled with these large granules. This change in position is probably brought about by the motion of the spore through the water, which crowds back the less dense cytoplasm, containing the large granules, from the forward end of the spore. Such a movement of the peripheral cytoplasm back, over, and around the denser cytoplasm results in a more clear-cut boundary of the dense cytoplasm in front of the nucleus. Figures 5 and 6 show spores which had been allowed to swim a number of hours at the optimum temperature. It will be noted that most of the cytoplasm containing the large granules has been crowded back or migrated away from the extreme anterior end of the zoöspore. The size of the zoöspores of the plants studied at Ann Arbor corresponds very closely to the measurements given by Barrett.

Saprolegniales

In this order the zoöspores are biciliate and either monoplanetic or diplanetic. From the point of view of zoöspore activity, three distinct groups of plants are included here: (a) the plants in which the zoöspores are active in both the first and second swimming stages, represented in this study by *Saprolegnia monoica* var. *glomerata*; (b) the plants in which the zoöspores may or may not be active during the first swimming stage but are always active after encystment. These fungi are represented here by *Isoachlya paradoxa*; (c) the plants in which the zoöspores are never active during the first swimming stage. The types used here were *Achlya conspicua* and *Aphanomyces euteiches*.

Saprolegnia monoica var. *glomerata*

The genus *Saprolegnia* was described by Nees von Esenbeck (33) in 1823. The species under discussion was described in 1858 by Pringsheim (36) and the variety in 1912 by Tiesenhausen (43).

Plants belonging to the genus *Saprolegnia* are very favorable material with which to work since they are easily collected, isolated, and carried in pure culture. They were therefore utilized for the perfection of much of the technique used in both the physiological and the cytological studies of the zoöspores.

Temperature and Oxygen Relations.—The studies of zoöspores of the species of the genus *Saprolegnia* have evidently been made by most students at whatever temperature happened to prevail in the room or culture at

the time that the observations were being made. No mention is made in the literature of an attempt to control accurately the temperature during zoöspore formation in these plants, and scarcely any clear-cut temperature data are available.

It was observed at various times by the writer that there is a relation existing between zoöspore activity and air, since zoöspores liberated or mounted in a hanging drop are more active and collect in greatest numbers near the surface of the drop where it is assumed they are able to absorb oxygen more readily. A number of experiments were therefore set up, in order to determine the effect, if any, that might be produced by the thorough aëration of the conductivity water prior to washing and mounting the young vigorous mycelium in it.

When two lots of mycelium, similar in all respects, except that one lot was washed and mounted in sterilized aërated water whereas the other was washed and mounted in sterilized but non-aërated water, it was found that the aërated culture formed and liberated zoöspores in one half to less than one half the time that was required by the non-aërated. Thus, at or near the optimum temperature for *Saprolegnia monoica* var. *glomerata*, spores would be liberated in well-aërated water after one and one-half hours. In the case of the non-aërated material three hours elapsed before any spores were liberated. Under less favorable temperatures but within the range of zoöspore formation, we get similar results, but the process takes a longer time in both aërated and non-aërated material. For example, at 26° C., one and one-half hours was the time required for aërated material and three hours for non-aërated material; at 22° C., three hours for aërated and six and one-fourth hours for non-aërated material; at 18° C., four and one-fourth hours for aërated and seven and one-half hours for non-aërated material. The results show that there is a very definite and consistent speeding up of the process of sporangial formation and zoöspore liberation when the material is well aërated.

The temperature range for zoöspore formation in *Saprolegnia monoica* var. *glomerata* ranges from below 16° C. to 32° C. The optimum temperature for zoöspore formation among plants belonging to this species lies very close to 26° C. At the optimum temperature sporangia are walled off one half hour after washing is complete and spores are beginning to be liberated one and one-half hours after completion of the washing process. The time required for the formation and maturation of the sporangia increased as the temperature at which the experiments were run was removed from the optimum. The most rapid increase of the time required for zoöspore formation is toward the maximum temperature.

Structure of the Zoöspores.—The gross morphology and ciliation of the zoöspores of species of *Saprolegnia* have been described repeatedly in the literature. The cytological details of the zoöspores however seem to have been almost completely neglected. De Bary (5) speaking of the cilia says,

"flagellum or one or two slender cilia with the power of lively motion spring from a definite spot in their surface as processes of the peripheral layer of protoplasm." Rothert (39) has shown that the cilia appear at first as slow outgrowths like little short straight bristles and that these show faint oscillations. No details of the relation to the organs of the cell, of either the young developing cilia or the mature active cilia, have been shown for members of this genus.

Each of the two cilia of the spores of the first swimming stage of this species has its insertion in a highly staining granule just beneath the plasma membrane at the smaller end of the spore. These basal granules seem to terminate the beak-like extension or extensions of the nucleus (fig. 14). That the main body of the nucleus is also well differentiated is shown in figure 14. A clearly defined dense chromatic body may be observed near the center of the nucleus in some of the spores. The central dense body is connected with the peripheral chromatin, which is situated just inside the nuclear membrane, by several very fine almost hyaline strands. Two of these strands uniting the central and peripheral chromatin seem to connect, one with either side of the base of the beak-like structure. The tip of this structure is terminated by the basal granules at the insertion of the cilia.

It will be noted that the nucleus is much nearer to the surface of the spore as represented in figure 15 than in the one shown in figure 14. The position of the nucleus as shown in figure 15 is the more typical one for the young spores. In the case of the older spores the nucleus has often moved back some distance toward the center of the cell. This condition is especially true of the zoöspore which is about to go into the encysted period following the first swimming stage. Spores were occasionally found in which the basal granules at the insertion of the cilia were somewhat wider apart than usual (fig. 16). It is evident from the spore represented in this figure that there are separate connections between the nucleus and each of the two basal granules. It is also evident that these connecting threads have their insertion on the main body of the nucleus in separate places which in this case are in definite chromatic granules which in turn are connected with a common chromatic mass. The chromatin in which each of the cilia have their final origin is in this case somewhat V-shaped with the tips of the V connected with the basal granules by definite strands.

The cytoplasm of these spores is divided into two rather distinctly differentiated parts. The central and more dense part surrounds the main body of the nucleus and extends through the central part of the body of the spore. There are numerous highly staining granules throughout this rather well differentiated central mass of cytoplasm. The peripheral zone of cytoplasm surrounds this central, more dense portion, and contains many small vacuoles. The peripheral cytoplasm is considerably more extensive in the anterior part of the spore body, causing an enlargement of this part of the spore; this results in the typical pear-shaped structure of these zoöspores.

Giant spores may be found occasionally (see general discussion for the physiological explanation of this phenomenon). Figure 17 shows one of these giant spores. Two nuclei are present, each, with its two cilia, having the typical organization of the species. These spores are very evidently the result of incomplete cleavage of the protoplasm in the sporangium.

As the spores emerge from their cysts, in preparation for the second swimming stage, the nuclear apparatus is observed to be considerably elongated and curved so as to appear somewhat crescent shaped. A granule may be seen at either tip. The tips of this curved nucleus approach or touch the plasma membrane. At a slightly later stage two finger-shaped pseudopod-like protuberances may be observed to have developed at points about as far apart as the distance between the tips of the curved nuclear apparatus and evidently at the places where the tips of the nucleus approached the plasma membrane (fig. 18). A somewhat later stage is shown in Plate XXXI, figure 19. The pseudopod-like projections have now become united and the one end of the nucleus has retreated somewhat from the granule which formerly was connected with it; this part or extension of the nucleus is beginning to round off and to be drawn back into the main body of that organ. In figure 20 we find the young cilia in the form of a loop, one side of which is much heavier than the other. There is also a small mass of colorless material which seems to have collected at the outer extremity of the loop. In figures 21 and 22 the cilia are observed to be almost mature. It will be noted that one is heavier, and in one case longer, than the other and that one is also more intimately connected with the nucleus. This may probably be explained by the unequal behavior of the nuclear apparatus in the very early stages of the development. The cilium which is most intimately connected with the nucleus probably developed in proximity to the basal granule, which did not become separated from the nucleus early in the cilia formation process, as did the basal granule of the other cilium.

The nucleus of the spore of the second swimming stage now becomes somewhat more definitely organized (figs. 23 and 24). The peripheral chromatin is often more definitely arranged toward the larger or anterior end of the nucleus (fig. 23). The cytoplasm of this, the second swimming stage, is divided into two distinct parts, an inner more dense portion and a peripheral less dense portion. The inner, more dense cytoplasm surrounds the nucleus and has irregularly pointed projections extending outward into the peripheral or less dense zone of cytoplasm. There are occasional vacuoles and some deeply staining granules found in this central cytoplasm. The peripheral cytoplasm is much less dense and contains many irregularly arranged vacuoles. The outer boundary of the peripheral cytoplasm in the younger spores is very irregular but becomes more regular as the spore matures. Occasionally, in preparations of these spores, deeply stained granules are found scattered through the peripheral cytoplasm.

Isoachlya paradoxa

This species was first described as *Achlya paradoxa* by Coker in 1914. Speaking of this plant he says, "so puzzling is the form that after preparing a description of it in 1912 it was decided to continue collections and experiments for another year before publication," and further, that "The difficulty arises from the fact that our plant combines in a most confusing manner the characters of both *Achlya* and *Saprolegnia*, and a rigid interpretation of these genera as at present defined would exclude it from both"; he concludes the paragraph by saying that "as the proliferation is usually of the *Achlya* type I have decided to refer this form to the genus *Achlya*." Kauffman (26) in 1921 described the genus *Isoachlya* and included this species along with others, all of which, he states, naturally fall within the boundaries of this genus. In 1923 Coker (9) erected the new genus *Protoachlya* for this single species. In speaking of this new genus he says, "It seems almost exactly intermediate between *Saprolegnia* and *Achlya*," and "The genus normally exhibits not only characters of several genera, but combines what has been considered antithetic characters, as both cymose and inter-sporangial proliferation of sporangia and motile and motionless spores on emerging." It is apparent from the description of the genus *Isoachlya* that it was formed to include plants of just such characteristics as those just quoted. From the studies described below, it becomes evident that the genus *Isoachlya* is broad enough to include all of the interesting variations of this species also.

Temperature Relations.—The optimum temperature for zoöspore formation and liberation for this plant lies between 23° and 25° C. The maximum temperature for this process lies between 32° and 34° C. Within the optimum range of temperature zoöspores are formed and liberated in from 4 to 6 hours. At 29° to 31° C., it requires from 10 to 12 hours for the completion of this process; a similar time is required at 16° to 18° C. Further lower limits for zoöspore formation for this species were not studied.

Under conditions near the optimum temperature for zoöspore formation it is found that there is but slight tendency toward the *Achlya* characteristics of this species. The spores under these conditions are expelled from the sporangium in a manner almost typically that of *Saprolegnia*. They do however, tend to hesitate, as it were, a short distance from the mouth of the sporangium seeming to wait for the entire group to become liberated from the sporangium. During this interval, of but a few seconds duration, the spores are in a state of constant agitation. They then swim away in the typical manner known for *Saprolegnia*. The secondary sporangia under these conditions are invariably formed by internal proliferation as in *Saprolegnia*. Spore formation in *Isoachlya paradoxa* may be quite unique. Where this occurs in a hanging drop preparation and when the conditions are as nearly optimum as possible, one can find in certain occasional

sporangia, spores which after liberation slowly creep and rock back along the wall of the sporangium and finally encyst there. Such a behavior is only observed in sporangia situated well on the interior of the small mass of mycelium of the mount, and occurs after other sporangia have been forming and liberating spores for a time. It would seem that this is probably the result of reduced aëration in this region of the mount.

By proper handling of the material dictyosporangia may be obtained in this species. These are produced experimentally by allowing the sporangia to develop just within the upper conditioned limit of zoöspore formation. They are then held for a few hours at a temperature just above this limit and finally brought to their optimum temperature. The formation of dictyosporangia under these conditions is explained by the fact that the spores which have been formed in the sporangia encyst there when placed under temperature conditions unfavorable for their being liberated. Then after again being placed under favorable conditions for spore liberation they are freed from their cysts through the old sporangial wall. When the spores have been prevented from escaping in a normal manner by being placed under somewhat unfavorable conditions the sporangiophores tend to form secondary sporangia as in the genus *Achlya*. The *Achlya* type of sporangial proliferation seems to be more prevalent on plants grown on flies than when developed from a young vigorous mycelium grown on dilute pea broth. Aside from the difference of constituents between the two food materials, in the case of the pea broth culture, food may be absorbed by any part of the entire mycelium, while in the case of the plants growing on the fly the major part of the nutrition must come from the attachment of the mycelium in the body of the fly. At any rate there is a marked difference noted between plants grown on the two food materials which emphasizes the importance of a standard substratum for this group of plants in order to be able to understand and interpret the various results reported by investigators.

Zoöspore formation and liberation as well as sporangial proliferation take place, under optimum conditions, in a manner almost typically that of *Saprolegnia*. If, however, plants are kept under conditions approaching the limits of zoöspore production, it is found that they tend to take on more of the characteristics of the genus *Achlya*. It seems that when the sporangia are produced under conditions which do not favor the prompt liberation of their zoöspores, the protoplasm of the hypha supporting the sporangium becomes active before the sporangium is emptied, resulting in the initiation of a lateral proliferation. It is apparent therefore, when studying the species of this genus which are even more sensitive to environmental changes than other members of the Oömycetes, that it is necessary to indicate clearly all of the conditions to which the plants are subjected. If this is done, then a genus such as *Isoachlya*, which also has characters of other genera, depending on the conditions under which the plants are allowed to develop, takes its proper place in plant classification.

Structure of the Zoöspores.—The cytological details of the zoöspores of these plants are similar in all respects to those described in detail for *Saprolegnia*. It seems therefore a waste of time and space to enter into a detailed discussion of the structure of the spores of this species.

Achlya conspicua

The genus *Achlya* was described by Nees von Esenbeck (33) in 1823. The species under discussion was described by Coker (9) in 1923.

Temperature Relations.—The optimum temperature for the formation and liberation of the zoöspores lies between 23° and 27° C., with slightly better indications between 25° and 26° C. At these temperatures sporangia are formed and spores liberated in from 4 to 5 hours. Sporangia are walled off in slightly less than 8 hours at 34° C. but no spores were observed to be liberated at this temperature. Spores are liberated in 6½ hours at 18° C. and in from 22 to 24 hours at between 10° and 12° C. The reactions at lower temperatures were not studied.

Structure of the Zoöspores.—In the developing sporangium at a stage during or somewhat later than the "homogeneous" stage of Büsgen (6), or the "stage of swelling of the spores" of Rothert (39), the nuclei may be observed to be in a state of activity. Humphrey (22) speaking of this stage says, "In spots corresponding approximately to the middle of the spore origins are to be seen clear, bright spots, and throughout the whole protoplasm are numerous vacuoles which appear and disappear shifting about rapidly." If a mass of these spore initials are killed and stained at this stage of development the nuclear apparatus may be observed to be in the form of a crescent-shaped spindle (fig. 25). The bulk of the chromatic material will be observed to occupy a position midway between the poles. At each pole, in favorably situated nuclei, may be seen one or more granules (fig. 25 *a*). In some of the spore initials these granules at the poles of the spindle have been brought into close proximity with the surface of the spore initial (fig. 25 *b* and *c*). These granules are the basal granules from the region of which the cilia develop. The poles of the nuclei and the accompanying granules are often at almost opposite sides of the spore initials (fig. 25 *c*). These granules apparently must migrate around toward each other until in the first swimming stage they occupy positions close together. Part of this migration evidently takes place after the cilia have started to develop. Couch (13), in agreement with Cornu (11) and Rothert (39), has shown that the spores of several species of this genus have connecting threads between the emerging spores. In the species studied by the writer such definite connections were found, at the same stage (fig. 26). Couch (13) in his discussion of these connecting threads states that "on other spores these threads were seen moving around from the opposite pointed ends of the spores approaching each other and on still others these threads had come to occupy the position of cilia."

The writer believes it to be a matter of chance whether the cilia happen to be connected with a thread in the process of their development. When spores are allowed to be formed under optimum conditions the cilia are quite immature at the time the spores are liberated from the sporangium (figs. 27 and 28). If we examine figure 28 it will be seen that the cilium (*a*) has developed relatively near to the attachment of the connecting thread (*b*). If we imagine this cilium developing directly underneath this connecting thread then in all probability the connecting thread might have developed into a cilium. The cilia which are at least partially developed at the time that the spores are liberated do not seem ever to develop sufficiently in this stage in this genus to function as organs of locomotion. They are soon absorbed as the spore prepares to enter the encysted stage. Figure 29 shows three spores which are preparing to enter the encysted stage. In the spore (*b*) it will be noted that the basal granules have been pulled toward the nucleus resulting in a drawing in of the plasma membrane. In the spore (*c*) the basal granules have broken away from the plasma membrane and have migrated some distance toward the nucleus. Figure 30 shows a spore in which the nucleus has almost reached a spherical shape as the spore enters the encysted stage.

The zoöspores of the second swimming stage of this species seem to be quite similar to those of *Saprolegnia* and *Aphanomyces*. The cilia are developed in the same manner as described and figured in some detail for these two genera. Figure 31 shows a spore in which the young cilia are almost free from the bit of debris to which the extreme ends still cling. Note the waves which evidently move out along the cilia from their base toward the tip. In the mature active spore the cilia may be observed to be inserted in basal granules which are connected with the nucleus (fig. 32). The cytoplasm surrounding the nucleus is more dense and less vacuolate than that nearer the periphery of the spore. Numerous larger granules may be observed in various parts of the cytoplasm.

Aphanomyces euteiches

The genus *Aphanomyces* was described by de Bary (3) in 1860. The species under discussion was described by Drechsler in Jones and Drechsler (24) in 1925 and has been shown by them to be the pathogen causing a serious root rot of peas.

Temperature Relations.—Jones and Drechsler report that the temperature range for spore liberation from the sporangial filaments extends from between 8° and 9.5° C. to between 33° and 35° C. and that the range of zoöspore activity extends from between 9° to 11° C. to between 21° to 22° C. At the lowest temperatures the zoöspores did not appear until after 49 hours or more time had elapsed. The optimum temperature for zoöspore formation and activity in this species was found by the present writer to lie between 26° and 28° C. As a matter of fact the range of

optimum temperatures for this process here really extends from 25° to 29° C. with slightly better indication between 26° and 28° C. The maximum temperature for spore formation was found to lie between 31.5° and 33° C. This it will be noted is a slightly lower maximum than was found by Jones and Drechsler. However the difference is readily explained by the fact that the writer did not extend his experiments on this point beyond 24 hours, since his chief interest was to find the optimum conditions for the production of normal typical zoöspores of the species. Some of the time relations to zoöspore formation at different temperatures as found by the writer for this species are as follows: at 17° to 18° C. more than 12 hours were required; at 19° to 21° C., 6 to 7 hours; at 21° to 23° C., 4½ to 5 hours; at 24° to 25° C., 4¼ hours; at 25° to 27° C., 4 hours; at 26° to 28° C., 3¾ hours; at 27° to 29° C., 4 hours; at 28° to 29° C., 4 hours; at 31° to 32° C., 7 hours; at 31.5° to 33° C., no zoöspores were formed.

The importance of favorable temperatures for the natural rapid development of *Aphanomyces euteiches*, in the presence of favorable moisture conditions, has been pointed out by Jones and Drechsler (24). In their investigation it was shown that an unusually rapid development of the root-rot caused by this fungus appeared following a period of five days during which time the mean daily soil temperature rose from 17.5° to 20.5° C. with the minimum for the period above 15° C. It will be noted that the soil temperature here reported approached to within less than six degrees of the optimum conditions for zoöspore formation and activity. At these soil temperatures zoöspores may be formed in from 6 to 7 hours. Since these soil temperatures are the mean average temperatures, in all probability the temperature during the warmer part of the day was very close to the actual optimum temperature. Since it requires less than four hours for the formation of spores at this temperature and since the soil temperature for the entire period was within the range of zoöspore activity, and provided there was a considerable quantity of mycelium present, the enormous possibilities of infection become apparent.

Structure of the Zoöspores.—The morphology of the first swimming stage of the forms of this genus and the discharge of the young spores from the sporangial filaments has been well described at different times by several botanists, viz. de Bary (5), Rothert (40), Couch (13), Jones and Drechsler (24). The writer is in agreement with these workers as to the fact that no cilia are found on the spores of the first swimming stage of this plant. Plate XXXII, figure 33, shows a spore which has recently emerged from the sporangial hypha and has rounded off in preparation for encystment. The thread-like appendages which formerly connected this spore with its neighbors may still be observed. It may also be noted that the nucleus occupies a central position at this stage of development.

This genus is particularly favorable for detailed studies of the development of the spores as they enter the second swimming stage. Since, under

favorable conditions, large numbers of spores are liberated through a single exit pore, and since they do not have any means of locomotion, they remain in close proximity to each other until after they emerge from their individual cysts. It is apparent then that we may have large numbers of spores emerging and going into the second swimming stage in a small area of the mount and over a short period of time. This enables one to have a wealth of material for study, very favorably located. The spores were allowed to develop in hanging drops which may be continuously observed, and killed and stained as desired.

Following the encystment of the zoöspores after their liberation from the sporangial filaments, the spore passes out of its cyst, the gross features of which have been well described by Jones and Drechsler (24). During or immediately following the process of emergence from the cyst the nucleus of the spore is observed to be the center of activity. The nucleus forms a spindle-like structure with several larger chromatic granules near the center and from one to three smaller granules near each of the poles. The entire structure is more or less curved, either crescent-shaped (fig. 34 *a* and *b*) or with two leg-like projections (fig. 34 *c*). In some cases a small pseudopod-like structure may be observed to project out from the surface of the spore opposite one or both poles of the nuclear apparatus (fig. 34 *a* and *b*). In figure 34 *a* all of the granules have been separated from one end of the spindle-like structure. This separation does not always occur, in fact it does not seem to be a usual occurrence at all as will be shown in the discussion of some of the later stages of these spores. The pseudopod-like extensions elongate very rapidly and by being lashed about often come into contact with some object to which they then adhere (figs. 35 and 36). The entire surface of the spore is quite irregular at this stage, numerous short protuberances being evident. This would seem to indicate that the entire peripheral part of the spore is in an active state. During the maturation of the cilia they seem to be in the condition of being stretched out and the granular elements, which had migrated out into them in the pseudopod-like stage, now migrate back toward the spore body and the cilia are soon torn loose from the foreign material with which they are in contact and the spore swims away. Figure 37 represents a spore which seems to have torn itself almost free from encysted spores with which its young cilia had come in contact. In some cases when the young cilia do not come in contact with another object they may stick together at their tips and by being lashed about they are stretched out in this loop-like manner (figs. 38 and 39) and are evidently finally torn apart so as to result in the usual two separate cilia. During the early stages of the maturation of the cilia the nuclear connections are not as evident as they are later, and the nuclear activity or migration of the granules seems to be away from the body of the nucleus. In the later stages, when the granular material seems to be moving back toward the body of the spore, the connections to the main

body of the nucleus become more evident. Occasionally the nuclear connections are much more pronounced toward the base of one cilium. An explanation of this is suggested by figure 34 *a* where the granules at one pole of the nuclear apparatus were separated at this early stage. Figure 40 shows a more mature spore in which one cilium seems to be entirely separate from the main body of the nucleus. The cilium is evidently controlled by the chromatic granules connected with the basal granule. These granules, it is assumed, were thrown off from the nucleus in a manner similar to that shown in figure 34 *a*. In many of the mature spores both cilia are found to be in direct connection with two parts of the nucleus (fig. 41). This is interpreted as a later development of the condition shown at an earlier stage in figure 34 *b* and *c* where both of the basal granules remained in contact with the tips of the nucleus at least until later in the development of the pseudopod-like projections of the cytoplasm. In many of the spores the opposite poles of the nucleus, where the granules were not dropped by one pole, have evidently moved around and united to form a single beak-like extension (fig. 39). In this case the basal granules often seem to have fused to form an elongated and curved chromatic rod connecting the two cilia at their bases and connected to the beak-like extension of the nucleus (fig. 39). There is a denser chromatic body which is situated in the beak-like extension and is connected with the basal granule by a definite strand. When there are two beak-like extensions of the nucleus as in figure 41 each has a denser chromatic granule; one, however, is likely to be much more massive than the other (fig. 41). The nucleus is shaped almost like an Indian club with the smaller end representing the beak (figs. 39 and 40) or when two beak-like structures are present it usually has the general shape as shown in figure 41. The cytoplasm of these spores is denser around the nucleus and gradually becomes less dense and more vacuolate toward the periphery of the body of the spore. There is a less clear-cut differentiation between the central and the peripheral cytoplasm of the spores of this plant than is found in the spores of *Saprolegnia monoica* var. *glomerata*.

Leptomitales

In this order the zoöspores are biciliate and either monoplanetic or diplanetic. From the point of view of zoöspore activity, this order includes two distinct groups of plants, (*a*) the diplanetic genera represented in this paper by *Apodachlya brachynema* and (*b*) the monoplanetic genera represented by *Rhipidium europaeum*.

Apodachlya brachynema

This plant was described in 1867 by Hildebrand (20) as *Leptomitum brachynema*. In 1883 it was placed in the genus *Apodachlya* by Pringsheim (37) when he described the genus.

Temperature and Oxygen Relations.—The optimum temperature for zoöspore formation lies between 15° and 19° C. Within this range sporangia are formed and zoöspores liberated in from 9 to 10 hours. Sporangia are formed in great abundance up to temperatures between 26° and 27° C. but zoöspores are rarely liberated above 22° C. Sporangia are formed at temperatures as low as 10° C. and spores liberated in considerable numbers at this temperature after 28 to 30 hours. In this species there is an unusually wide range within which sporangial formation takes place above the temperature limit for zoöspore liberation.

Considerable difficulty was experienced at first in obtaining spores of the second swimming stage. It was found, however, that after spores of the first swimming stage have come to rest and are completely encysted the second swimming stage can be initiated by replacing the water of the hanging drop of the mount with water that has been thoroughly aërated. The water which is used to replace the vitiated water of the mount should be aërated at or below the temperature of the mount. Within the optimum range of temperature encysted spores treated with newly aërated water will begin to emerge vigorously from their cysts at the end of one hour.

Structure of the Zoöspores.—The zoöspores of the first swimming stage of this species exhibit the most uniform and characteristic ovoid shape of any of the biciliate spores studied. These spores are nearly symmetrical, usually, however, with a slightly flattened area on one side near the insertion of the cilia, giving to the spores a slightly lop-sided appearance. The cilia are two in number, comparatively short, each having its insertion in a separate basal granule. The basal granules are connected with the tip of the nucleus by definite thread-like structures. The nucleus is oval in shape with an almost beak-like tip in which may be found the denser chromatic body which is connected with the basal granules. The cytoplasm is very finely granular and contains numerous small vacuoles; the latter are more prevalent in the peripheral and anterior part of the cell (Pl. XXX, fig. 7).

The spores of the second swimming stage are ovoid in shape. The two cilia are inserted laterally in definite basal granules; the latter are either separate (fig. 7) or appear to be fused (fig. 8). Definite connections may be observed between the basal granules and the nucleus. The nucleus also is ovoid in shape and irregular chromatin granules are distributed in its peripheral region. Occasional spores may be observed with the nuclei slightly curved (fig. 10). Near the tip of the nucleus larger and denser granules are usually evident at the insertion of the strands connecting this organ with the basal granules. The cytoplasm is very finely granular, with occasional very small vacuoles. In some spores the peripheral region of the cytoplasm is found to contain many granules slightly coarser than those of the central cytoplasm (fig. 8).

Rhipidium europaeum

The genus *Rhipidium* was described by Cornu (10) in 1871. His original species were *R. continuum* and *R. interruptum*, which were connected by von Minden (32) and probably conceived by him to be equivalent to *R. europaeum*.

Temperature Relations.—No reference is made in the literature to any observed temperature relation to the formation and liberation of zoöspores in the species of this genus. Scarcely any illustrations of the liberated zoöspores of these plants are found among the various illustrations of plants belonging to this genus. One naturally wonders regarding this dearth of illustrations of an important stage in the life history of an entire genus of plants. From what follows below, it is easy to infer that such descriptions and illustrations of these plants as are found in the literature have probably been made from plants observed under conditions for zoöspore formation other than the optimum (Minden, 32, and Myk. Untersuch. und Ber. 2: 146-254. 1916; and Thaxter, Bot. Gaz. 21: 317-331. 1896).

The temperature range for the formation of zoöspores and their liberation in this plant is from about 12° C. to slightly above 22° C., a total range of but ten degrees centigrade. The optimum temperature for zoöspore formation and activity lies between 16° and 18° C. The fairly low temperature range of zoöspore formation and liberation is probably the explanation for the dearth of illustrations. If plants are kept at near the maximum temperature for this process, under rather unfavorable conditions of aëration, zoöspores are formed but not liberated. This emphasizes the importance of complete control of environmental conditions when studying the life history of these highly sensitive plants.

Structure of the Zoöspores.—The motile spores of this monoplanetic plant exhibit the same general form and structure as those found in the second swimming stage of related genera. There are, however, some characteristic details, serving to distinguish the spores of this plant, which will be discussed as we study the details of the different parts of the motile spores. The cilia have their origin in a single granule or a complex of basal granules (fig. 11). The granules are in either case united with a more massive chromatic structure situated adjacent to the nucleus. The nucleus in the more mature spore, when viewed from the side, has much the same organization as that described elsewhere in this paper for the spores of the second swimming stage of *Saprolegnia monoica* var. *glomerata*. The cilia may continue to be connected with the almost opposite ends of the nucleus (fig. 12) or the cilia, along with the basal apparatus, may have migrated around to a lateral position near to each other (fig. 13). The peripheral chromatin in the nucleus of a spore, with its parts oriented as in figure 13, when viewed in optical section, appears to be shaped almost like a horseshoe, with the toe of the shoe occupying the position of the broader or anterior part of the nucleus. The mass of chromatic material

near the tip of the nucleus is somewhat curved, and the ends of the curve point toward the basal granules at the insertion of each of the two cilia. This chromatic material is evidently a product of the fusion of two masses similar to those shown at the base of each ciliary apparatus in figure 11.

The central cytoplasm surrounding the nucleus is less extensive than that found in the species of *Saprolegnia* studied. The peripheral cytoplasm on the other hand is more extensive than was found in other zoöspores. There are many very regular and clear-cut vacuoles distributed throughout this region of the cell. They are usually arranged in a single layer just inside the plasma membrane. The framework of cytoplasm filling the spaces between these vacuoles seems to be of a denser nature than that found in the peripheral region of the other biciliate spores. Cytoplasm filled in this fashion with a rather regular array of vacuoles gives to these spores a coarse, rough appearance, even when observed in the active living condition.

Peronosporales

In this order the zoöspores are biciliate and monoplanetic. The order is represented in this study by five species of the genus *Phytophthora*; one species, *P. palmivora*, is studied cytologically.

Phytophthora

The genus *Phytophthora* has attracted the interest of mycologists and pathologists since its description by de Bary (4) in 1876. This interest is probably due first to the economic significance of the various species of the genus, and second because the critical separation of the species of this genus is not promoted by the presence of sharp morphological characters.

Temperature and Aëration in Relation to Zoöspore Formation.—Among the studies reported in the literature regarding the effect of various degrees of temperature on the formation and activity of the zoöspores of this genus are those of Dastur (14) who has shown that in *P. parasitica* Dastur the zoöspores are liberated from sporangia in five minutes at 25° C. and that higher temperatures retard the formation of zoöspores. Melhus (31) who studied zoöspore formation in *P. infestans* (Mont.) de Bary, has shown that the optimum temperature for zoöspore formation in this species lies between 12° and 13° C., with the minimum between 2° and 3° C., and the maximum between 24° and 25° C. Gadd (16), in his work with *P. faberi* Mont., has shown that the optimum temperature for zoöspore formation in this species lies between 20° and 25° C. and that zoöspore formation is retarded below or above these temperatures. He has also shown that well-aërated water is necessary for the formation of zoöspores in large numbers. Ashby (1) working with *P. palmivora* says, "When the mature sporangia from cultures five to seven days old were brought into cool well-aërated water, a number showed segmentation of the granular contents and discharged zoöspores within fifteen to thirty minutes."

The complete temperature range for zoöspore formation was obtained by the writer in the study of an undescribed species of *Phytophthora* obtained from Dr. D. Reddick. This species, along with *P. palmivora*, *P. terrestris*, and others, has been combined into de Bary's *P. omnivora*, in the studies of this genus reported by Leonian (29). This procedure seems to be questionable in the light of our far from complete knowledge of the group. The temperature range of zoöspore formation in this species extends from between 4° and 6° C. to between 34° and 36° C. with the optimum between 25° and 27° C. At the optimum temperature spores are liberated in well-aërated water in five minutes. At temperatures removed from the optimum the time required for zoöspore liberation increases to an hour or more near the limits of zoöspore formation for the plant.

In *P. erythroseptica* the relation of zoöspore formation to aërated and non-aërated water was also studied. The temperature relation was found to be very similar to that just shown for Reddick's *Phytophthora*. It was found that at the optimum temperature spores were liberated in five minutes when using well-aërated water and were not liberated until after more than three hours when using non-aërated water.

The plants of *P. cactorum* and *P. terrestris* showed reactions similar to those of the species just discussed, excepting that the minimum time for zoöspore formation for these two species was found to be ten minutes instead of five minutes.

The writer has found that the optimum temperature for zoöspore formation in *P. palmivora* lies between 23.5° and 25.5° C., at which temperatures zoöspores were liberated in nine to ten minutes. As the temperature at which the preparation is kept is gradually moved from the optimum, there is an increase in the time required for the formation of zoöspores. Thus between 25.5° and 27° C., it requires seventeen to eighteen minutes for the liberation of spores; between 27° and 29° C., it requires twenty minutes; between 29° and 31° C., it requires from thirty to sixty minutes. Above 31° C. zoöspores were not formed.

In view of the fact that the temperature range for zoöspore formation is distinctly different in this species and that its optimum falls below the optimum for Reddick's *Phytophthora* and for *P. terrestris*, and since these species also have morphological and pathogenic differences which cannot be disregarded, it becomes evident that the identity of the species of this genus cannot be based on any one set of characters either morphological, physiological, or pathogenic. It also becomes evident that in order to understand plants which are so sensitive and are so difficult to separate into specific groups by their morphology alone, the worker must be thoroughly acquainted with all their specific reactions under controlled conditions.

Structure of the Zoöspores of P. palmivora.—The size of the spores of the plants studied corresponds to the measurements given by Butler

in his description of the species (Mem. Dept. Agr. India Bot. Ser. 1, 5: 1-60. 1907). The spores are kidney-shaped or ovoid with a flattened space on one side where the cilia appear. This is known to be the characteristic shape of the zoöspores in this group. There are, however, many spores which vary all the way from kidney-shaped to a nearly spherical structure, usually, however, with a somewhat flattened dent at the insertion of the cilia. At optimum conditions the cilia are two in number and usually have their insertion in a definite chromatic body, the basal granule, which in turn is connected with the nucleus (Pl. XXXII, fig. 42). In some of the spores the cilia appear to be connected directly to the nucleus; in such cases it is impossible to differentiate the basal granules (fig. 44). In certain spores only one cilium could be found to have definite nuclear connections, the other having its origin in a basal granule; the latter was somewhat larger than that found where the basal granule was connected with the nucleus by a definite strand. There are also occasionally triciliated spores in which the basal granule of one cilium is definitely connected with the nucleus while the other two cilia have their insertion in separate basal granules, with which there was no apparent connection with the nucleus or with each other (fig. 43). The formation of extra cilia and of cilia with basal granules entirely separated from the nucleus, is interpreted as being the result of the basal apparatus at one pole of the nucleus becoming separated from the nucleus early in the process of cilia formation. This is probably the result of an earlier condition similar to that described for the early stage of the spores of *Aphanomyces euteiches* and shown in figure 34 *a*. This triciliated condition with two of the cilia inserted on basal granules entirely separate from the nucleus would seem to indicate more definitely that the basal granules are directly responsible for cilia formation, and possibly also for the later control of the activities of these organs.

The cytoplasm of these spores is very finely granular. The more dense portion surrounding the nucleus is quite limited in some cases as in the spore shown in figure 44. In other spores this central zone of cytoplasm is more extensive (fig. 42). There are occasional larger, more densely stained granules scattered through the cytoplasm. These granules are more prevalent in the central than in the peripheral cytoplasm. The peripheral cytoplasm is less dense and is filled with many small vacuoles which give to this part of the cell a rather loose appearance.

GENERAL DISCUSSION

Influence of External Conditions

The importance of the influence of external conditions on the ability of various species of the Oömycetes to produce the different phases of their life cycles has been shown by a number of investigators including Klebs (27), Kauffman (25), Pieters (35), and others. Klebs was the first to show that zoöspore formation among the Oömycetes is initiated when a well-nourished

mycelium suddenly experiences a lack of food, as, for example, when it is thoroughly freed from an external food supply and then transferred to pure water. It is evident from the work reported in this paper that, even though a plant may have a wide range of conditions under which zoöspore formation is initiated or partially stimulated, there are definite narrow limits of temperature and aëration, and the maximum number of zoöspores will be formed only within such limits. It is seen, further, that the typical zoöspores are produced only from a vigorous, well nourished mycelium from which the food has suddenly been thoroughly removed. In other words the removal of the food from the young active mycelium sets in motion the potential ability to form sporangia and zoöspores while the completion of this process, especially in a manner typical of the species, depends on favorable conditions of temperature and aëration. Temperatures above the optimum and poor aëration, conditions which are unfavorable to the process, often result in incomplete cleavage of the protoplasm in the sporangium. Such conditions may completely inhibit zoöspore formation or cause the formation of giant spores. In a preparation which was well-aërated at the beginning, loss of oxygen occurs more rapidly at the higher temperatures. This is due to the fact that, at these temperatures the oxygen in the preparation is used up, and the products of metabolism are liberated faster than at lower temperatures. The liberation of carbon dioxide is frequently used as an index to these activities. Kostychev (28) has stated that "the amount of carbon dioxide given off increases regularly with the gradual increase in temperature, finally reaches a maximum value and remains at the same level until the death of the plant from the high temperature (at about 50°)." It is apparent then that when plants with a low optimum for zoöspore formation, as for instance *Blastocladia Pringsheimii* or *B. globosa*, are studied under ordinary room temperatures which range from 6° to 10° C. higher than the optimum, the zoöspores are quite likely to be atypical. In plants where the optimum temperature for this process falls near to or within the range of ordinary room temperatures as in *Saprolegnia monoica* var. *glomerata* or *Isoachlya paradoxa*, giant spores due to incomplete cleavage are only occasionally liberated and then only in the interior of the mount where the sporangia are smothered. This is more likely to occur when too much mycelium is used in the preparation of the mount, and is interpreted as being due to poor aëration in this part of the mount.

Cilia Formation in Uniciliate and Biciliate Groups

The question naturally arises as to just what are the cytological, physiological, physical, or chemical differences between the uniciliate and the biciliate groups at the time of zoöspore formation. One may ask, are there any observable details that may be pointed out to account for the fact that in one group the spores are normally biciliate while in the other

group we find the uniciliate condition to be characteristic of the motile spores? In the case of the second swimming stage of the diplanetic species, during and immediately following the process of freeing themselves from the resting cysts, a marked increase in size is noticed. This is brought about by the rapid absorption of water along with the formation of many vacuoles in the peripheral part of the cytoplasm. The cilia are formed by these spores during the period when water is being absorbed with an accompanying increase in size. It is not definitely known, on the other hand, at what exact stage the cilia are formed in the maturation of the spores in the sporangia. Davis (15) in his study of the young zoösporangium of *Saprolegnia* has shown that the nuclei are not active before or during the cleavage of the protoplasm into uninucleate spore initials and are not ciliated at this time (Davis, 15, figs. 30-35). There is further evidence that the cilia are often not completely matured when the spores are liberated from the sporangium (Couch, 13). The writer has shown that cilia formation is initiated in the sporangium of *Achlya* after cleavage of the protoplasm into spore initials (page 526). However, the cilia are not formed immediately after cleavage in this biciliate group of plants but probably immediately prior to the liberation of the spores from the sporangium.

Barrett (2) in his study of *Allomyces* has shown that the nuclei are evidently concerned in the early process of cilia formation during or soon after cleavage has taken place. In his figure 31, it will be noted that the beak-like extensions of nuclei have formed and are in some cases in contact with the newly formed plasma membrane. Speaking of this figure Barrett says, "Fig. 31 represents a part of a section of a sporangium in which the segmentation is almost complete. . . . The limiting surfaces of the spore masses in a number of cases have separated. . . . Apparently contraction has taken place, which would indicate that the mature spores occupy less space than the original masses of protoplasm from which they were formed." It seems apparent that cilia formation in this species is initiated during or immediately following the cleavage process and is probably immediately preceded by nuclear division, since Barrett has shown that nuclear division does take place in the sporangium prior to cleavage. Jahn (23) in her studies of nuclear division and cilia formation in the zoöspores of *Stemonitis flaccida* Lister, has shown that cilia formation accompanies nuclear and cell division in this case. It thus becomes apparent that, in the spores of at least a number of the species of the uniciliate groups of fungi, cilia formation accompanies or follows closely nuclear and cell division. In these cases a single cilium is initiated or formed at each pole of the mother nucleus, which finally results in the presence of a single cilium for each of the daughter cells or zoöspores of the species.

On the other hand, in certain fungi which have under optimum conditions biciliate zoöspores, it is known that cilia formation does not accompany or closely follow nuclear or cell division. In the spores in the second

swimming stage of species of *Saprolegnia*, *Achlya*, and *Aphanomyces* cilia formation is preceded or accompanied by nuclear activity and spindle formation. The cilia are formed as a result of activities centered in certain granules which occupy or formerly occupied a position at the poles of the nucleus. During this process it should be noted that instead of cell division there is a marked increase in size of the protoplast accompanied by the absorption of water and the formation of many small vacuoles in the peripheral cytoplasm. This it will be noted is exactly the opposite of what takes place during the process of cleavage of a mass of protoplasm, as has been shown by Hofmeister (21) who pointed out that the division of the protoplasm in the process of cleavage is accompanied by loss of water, increased density, and reduction of volume. Harper (18) states that "in the gradual shrinkage and condensation of the spore-plasm the loss of water might be least in the neighborhood of the nuclei and that thus a determining factor in the orientation of the cleavage surfaces would be introduced." Swingle (42) says that "the most probable explanation the writer has found for the mechanics of cleavage is on the basis of local contractions of the cytoplasm, somewhat comparable to the phenomenon exhibited in the naked protoplasm of amoebae and pseudopodia." It is apparent then that cilia formation in the spores of the uniciliate and of the biciliate species of fungi takes place under entirely different internal conditions. The initiation of cilia formation takes place during very early stages of spore formation in the uniciliate group. In the biciliate group this process is delayed until later when greater nuclear activity develops; the latter is sufficient to bring about cilia formation but the conditions present do not permit of further cell division. Back of all this, of course, lies the different genetical make-up of the species in the two groups.

Structure of the Basal Apparatus and its Relation to the Nucleus

The status of our knowledge concerning the basal apparatus as found in various ciliated cells has been adequately discussed in the work of Wilson (45) and of Sharp (41). The writer therefore will not review this material, but refers the reader to those authors for the general discussion of points bearing on this subject.

Hartman (19) has shown that in *Eudorina elegans* the pointed end of the nucleus to which a "centriole" is attached, touches the plasma membrane and then retreats leaving behind a double body from which the cilia grow. This description of the formation of the cilia in *Eudorina elegans* seems to be the nearest approach, of anything described in the literature, to what happens during cilia formation in the zoöspores of the fungi studied by the writer. In the zoöspores, especially of the second swimming stage of species of *Achlya*, *Aphanomyces*, and *Saprolegnia*, the writer has shown, however, that both poles of the nucleus, with their attached or included granules, approach very near to or actually touch the plasma

membrane; they may then withdraw from the membrane leaving there one or more granules. At points near these granules we find the cilia appearing, first as pseudopod-like projections, which later are matured into active cilia. The question now arises, are these basal granules, which seem to control the development of cilia in the zoöspores of the Oömycetes, homologous with the basal apparatus of the protista and those found in the spermatogenesis of some animals and plants? Wilson (45) has said that "the relations between these various basal structures have not yet been completely elucidated."

It would seem, however, that the basal apparatus in these fungi is very definitely of nuclear origin. In the development of the spores of the second swimming stage of *Achlya conspicua*, *Aphanomyces euteiches*, and *Saprolegnia monoica* var. *glomerata*, it is shown that these granules, often three in number for each cilium, are first found occupying positions near the poles of the crescent-shaped nuclear apparatus as the spore prepares for cilia formation. If the granules at one pole of the nucleus become completely separated from the nucleus early in the process of cilia formation, but remain in close proximity with each other, they may under certain conditions remain separate from the nucleus and have complete control of the development and function of one cilium (*Aphanomyces*, fig. 40). If, however, these granules become somewhat more separated from each other, a separate cilium may be developed from each granule (*Phytophthora*, fig. 43). It is apparent, then, that even though these basal granules are shown to be of nuclear origin they may develop and control cilia after, in so far as one can see, they have been completely separated from the nucleus. This would seem to show that the cilia of the spores in these fungi may have their origin and may be controlled by differentiated parts of the nuclear material and not by the entire organ.

In the majority of cases of normal spore development of the second swimming stage both cilia are found to remain attached to the nucleus. They or one of them may have migrated away from the pole or poles of the nucleus so as to occupy a position on one side of that organ. This position probably has something to do with the usual shape of the spores at this stage. There are definite, more dense chromatic bodies inside the nucleus in which the strands (rhizoplasts) running from the basal granule to the nucleus have their origin. The writer is inclined to believe that normally but one granule is dropped from each tip of the nuclear apparatus, and that the remaining granules near the tip form this denser chromatic body. When the basal granules along with their cilia have migrated fairly close together these denser chromatic bodies often seem to fuse forming a larger somewhat curved body (*Rhipidium*, fig. 13). In the first swimming stage of the diplanetic species this chromatic body at the insertion of the rhizoplasts in the nucleus is often somewhat V-shaped. In very favorable material a granule may be found near each leg of the V to which the rhizo-

plast is attached. The base of the V is evidently formed by the fusion of two or more granules which formerly formed the extreme base of the rhizoplast from each cilium (*Saprolegnia*, fig. 16).

It is therefore apparent that, in the zoöspores of the fungi here discussed, the basal granules are of nuclear origin. The basal apparatus is composed of several granules, usually three in number, two of which usually remain within the nucleus and form the denser chromatic body in which the connecting strand or rhizoplast has its insertion in the nucleus. This body under certain conditions may apparently fuse with the similar body of the basal apparatus of the other cilium forming the larger body described above. The other granule forms the basal granule proper, or "blepharoplast," at the base of the cilium.

The Encysted Spore Stage of Diplanetic Species

A number of interesting questions arise in connection with the encysted stage of the zoöspores of the diplanetic species of the Oömycetes. Why should such encystment be interpolated between the two swimming stages? There is also the question of the relation of the spores of the second swimming stages of these plants to spores of similar morphology and cytology of monoplanetic species. It is evident that an adjustment of the nuclear apparatus takes place during or immediately following the encystment. This results in morphological changes which are present in the second swimming stage. There is also a marked change in the physiological reactions of the spore of the second swimming stage and this is probably controlled by this nuclear adjustment. The spore is now able to function as a reproductive body. It is now able to absorb food and initiate the process of growth resulting in the production of a vegetative mycelium. Apparently it could not accomplish this process before this nuclear and the accompanying physiological adjustment had taken place. The behavior of the zoöspores of some related groups which have but one swimming stage and which resemble in structure and function the spores of the second swimming stage of the diplanetic species, may be explained by the fact that this nuclear adjustment in the monoplanetic species has taken place before liberation from the zoösporangium. It is not difficult to believe that the *Achlya* type of diplanetic spores, where cilia formation in the first swimming stage is initiated but usually not completed, and *Aphanomyces*, where there is no indication of cilia formation in this stage, are but a step removed from the condition where this nuclear and physiological adjustment takes place in the sporangium.

Ciliation a Constant Character

There is one other important relation brought out by this work which should be emphasized. The writer refers to the importance of the ciliation of the zoöspores in connection with the classification and possible phylo-

genetic connections of the group. The writer (12) has pointed out in his studies of the genus *Blastocladia*, that "the cilia are constant in number and position when allowed to develop normally under conditions most favorable for zoöspore formation." The present work makes it possible to apply this statement to a much larger group of the Oömycetes if not to the entire sub-class. Even "giant zoöspores"⁴ show the characteristic number of cilia in relation to each nucleus when properly stained and cleared. It is apparent then that ciliation of the zoöspores of the Oömycetes is a stable character when the spores are studied under favorable conditions and properly stained. The number of cilia of the zoöspores may now be recognized as it has been by some botanists as one of the fundamental characteristics of these plants. It is a useful character in connection with the classification of the larger groups of the Oömycetes, and in any considerations dealing with the phylogeny of the group.

SUMMARY

1. In order to obtain zoöspores of the Oömycetes at will, in abundance and in normal condition, so that they may be studied in any stage of development desired, optimum conditions of temperature and aëration must first be thoroughly controlled by the experimenter.

2. For each species there is a certain definite set of conditions under which it is capable of forming most abundantly perfect zoöspores typical of the species. Any single factor or set of factors, such as temperature, aëration, and food supply influences the formation of sporangia or of zoöspores.

3. The optimum temperatures for the formation of zoöspores were determined in the following species: *Allomyces arbuscula*, between 25° and 27° C.; *Saprolegnia monoica* var. *glomerata*, very near 26° C.; *Isoachlya paradoxa*, between 23° and 25° C.; *Achlya conspicua*, between 25° and 26° C.; *Aphanomyces euteiches*, from 26° to 28° C.; *Apodachlya brachynema*, between 15° and 19° C.; *Rhipidium europaeum*, between 16° and 18° C.; *Phytophthora cactorum*, *P. erythroseptica*, *P. parasitica*, *P. terrestris*, and *Phytophthora* sp. between 25° and 27° C.; *Phytophthora palmivora*, between 23.5° and 25.5° C.

4. A technique has been developed by which zoöspores may be so stained as to demonstrate the basal apparatus of the cilia and its connection with the nucleus. This technique involves killing with fumes of osmic acid, staining with crystal violet, and drying of the preparation (over sulfuric acid), followed by the usual procedure of clearing and mounting.

5. The zoöspores of the species studied all have a definite basal granule

⁴ In a paper which came to hand after the present paper was completed, Hans Kniep (Ber. Deutsch. Bot. Ges. 47: 199-212, 1929) has shown, in his study of *Allomyces javanicus* n. sp., that "giant zoöspores" occur in his species of *Allomyces* similar to those reported by the writer (12) for *Blastocladia globosa* and *B. pringsheimii* and in this paper for *Saprolegnia monoica* var. *glomerata*; both Kniep and the writer have interpreted the phenomenon in the same way.

(blepharoplast) at the insertion of each cilium. This granule is connected with the nucleus by a definite strand (rhizoplast).

6. The cilia develop as outgrowths from the region of a basal granule which has reached a position near the plasma membrane as a result of nuclear activity.

7. The basal apparatus is always of nuclear origin and seems to be composed of several chromatic bodies, one of which forms the basal granule proper (blepharoplast). The remaining granules of the basal apparatus are normally retained or drawn back into the nucleus and usually form the tip or beak-like part of that organ during zoöspore activity.

8. When the zoöspores of either swimming stage come to rest, the basal granule or granules, as the case may be, migrate back to the nucleus and apparently become part of that organ.

9. The chromatin of the nucleus in the mature active zoöspores of these fungi is arranged in irregular masses in the peripheral region. In some spores a larger central mass of chromatin may be distinguished near the center of the nucleus.

10. Temperature conditions away from the optimum for a species have been shown to be the cause of certain abnormalities hitherto described in the literature, as for example variations in the number of cilia or the presence of "giant" spores.

11. The results obtained by the study of such a large number of examples from every group of the Oömycetes have emphasized the phylogenetic value of the cilia because of their constancy in a genus.

The studies reported in this paper were completed in the Botanical Laboratory of the University of Michigan under the direction of Dr. C. H. Kauffman who suggested the problem and to whom the writer wishes to express his appreciation for the many helpful suggestions made and the keen interest shown during the progress of the work and the preparation of the manuscript.

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DESCRIPTION OF PLATES

All drawings were made to the same scale, with the aid of a 2 mm. oil immersion objective, a 12 X compensating ocular, and a camera lucida.

PLATE XXX

FIGS. 1-6. *Allomyces arbuscula*

FIG. 1. Young zoöspore with immature cilium, note basal granule with 3 smaller granules connected with it, and situated adjacent to the nucleus. The chromatin of the nucleus is distributed around the periphery and in a central mass which in turn is connected with the basal apparatus. All of the deeply stained granules in the cytoplasm are situated in the distal region of the spore.

FIG. 2. A somewhat older spore in which some of the deeply stained granules have been moved toward the ciliated end of the spore. The three-parted basal apparatus is also evident in this spore.

FIG. 3. The granules of the basal apparatus nearest the nucleus have migrated together, apparently forming a single larger body which now forms the tip of the nucleus. The dense mass of cytoplasm in front of the nucleus is beginning to be crowded back into a rather regular oval mass almost enclosing the nucleus.

FIGS. 4-6. Different stages in the orientation of cell structures due to activity of the spore; note change in position of deeply stained cytoplasmic granules in spores 3, 4, 5, and 6.

FIGS. 7-10. *Apodachlya brachynema*

FIG. 7. First swimming stage, note individual basal granules with separate nuclear connections.

FIGS. 8-10, second swimming stage. FIG. 8. The basal granules are apparently fused, but separate nuclear connections may be seen. The peripheral cytoplasm shows many small deeply stained granules.

FIG. 9. The separate basal granules for each cilium may be distinguished in this spore.

FIG. 10. Spore in which the distal part of the nucleus has not become completely rounded off following the formation of the cilia.

FIGS. 11-13. *Rhipidium europaeum*

FIGS. 11 and 12. Spores in which the cilia remain attached to opposite parts of the nucleus before migrating to one side; note regular arrangement of the vacuoles in the peripheral cytoplasm.

FIG. 13. Side view of a spore in which the cilia along with each basal apparatus have become oriented on one side of the nucleus.

FIGS. 14-24. *Saprolegnia monoica* var. *glomerata*

FIGS. 14-17, first swimming stage. FIG. 14. Large spore with the basal granule for each cilium well differentiated. The nucleus is nearly round with a central mass of chromatin which is connected with the basal apparatus. The cytoplasm is finely granular and more dense in the central region and more vacuolate towards the periphery; occasional deeply stained cytoplasmic granules may be seen.

FIG. 15. A smaller spore with the nucleus somewhat over-stained. A vacuole is evident in the distal part of the nucleus.

FIG. 16. Spore with the strands connecting the basal granules with the nucleus crossed, showing their origin in this case to be in separate granules inside the nucleus, these granules being connected each with one leg of a V-shaped mass of chromatin. The central cytoplasm shows many deeply stained granules.

FIG. 17. A giant spore with two nuclei each characteristically biciliate.

FIGS. 18-24, second swimming stage. FIG. 18. Bristle-like projections of the cytoplasm shortly after initiation of cilia formation, somewhat over-stained.

PLATE XXXI

FIG. 19. A slightly later stage with the nucleus near the surface of the spore. The basal granules may be seen near the tips of the nucleus.

FIG. 20. The young cilia have touched and adhere to each other at the tip.

FIGS. 21, 22, and 23. Relatively young spores. Note that the base of each cilium is enclosed in a cytoplasmic matrix; the nuclear connections (rhizoplasts) are less evident at this stage than in spores which have been active for a longer time.

FIG. 24. Side view of spore which has been active for considerable time. Note that connections between basal granules and nucleus are quite distinct, each basal apparatus in this spore has a separate attachment in the nucleus. The cytoplasm is divided into a central more dense and a peripheral more vacuolate region.

FIGS. 25-32. *Achlya conspicua*

FIG. 25. Spore initials showing crescent shaped nuclei, *a* with one pole, *b* and *c* with both poles with included granules approaching the plasma membrane.

FIG. 26. Two of a group of spores before emerging from a sporangium showing connecting strand.

FIG. 27. Spore which was not attached to others in sporangium which was in process of being emptied. Note initiation of cilia formation with granules near the tip of the finger-like projections.

FIG. 28. Spore just liberated from sporangium. Note two short cilia, one of which, *a*, lies near an old connecting thread, *b*; the nucleus appears to have been folded together with each basal apparatus remaining attached to each tip.

FIG. 29. *a*, young cilium has been absorbed but basal granules are still near surface of spore; *b*, basal granules apparently pulling plasma membrane slightly toward nucleus; *c*, basal granules free from plasma membrane have migrated almost to the nucleus.

FIG. 30. Spore going into encystment. Note nearly spherical nucleus.

FIG. 31. Young spore of second swimming stage, cilia nearly ready for use in locomotion. Note attachment of tips to piece of debris, and waves in the cilia when the spore was killed.

FIG. 32. *a*, spore of second swimming stage which has been active. The basal granules appear to be fused forming a rod-shaped body which seems to be attached to the nucleus by a single strand. Note two distinct zones of cytoplasm and the presence of many deeply stained cytoplasmic granules; *b*, similar to spore (*a*) except that basal granules are separate and distinct.

PLATE XXXII

FIGS. 33-41. *Aphanomyces euteiches*

FIG. 33. Spore which has just emerged from the sporangium, the connecting threads are still evident.

FIG. 34. *a*, spore just out of encystment. Note granules which have been dropped from one tip of the nucleus, and included granules in other tip; *b*, spore with 3 granules in each tip of the nucleus. Note a single granule at each extreme tip or pole of the nucleus; this is evidently the young basal granule proper (blepharoplast); *c*, spore showing nucleus with two leg like projections. Note granule in tip of each.

FIGS. 35, 36, 37, and 38. Various stages in the development of cilia a short time after the spores have emerged from the encysted stage. Note the loop-shaped form of the young cilia, with granules variously distributed at first, and later mostly concentrated at the base of the cilium. In figs. 37 and 38 the cilia are approaching maturity, and are nearly free from the debris to which they cling.

FIG. 39. Spore with cilia still attached to each other, but evidently in last stages of freeing the tips of the cilia.

FIG. 40. Mature spore in which the one basal apparatus was evidently completely dropped by the nucleus, probably as in fig. 34*a*.

FIG. 41. Active spore with cilia connected with different parts of the nucleus. Note basal granules, and denser body inside the nucleus at the insertion of each connecting strand. The cytoplasm is finely granular, somewhat more dense towards the center near the nucleus.

FIGS. 42-44. *Phytophthora palmivora*

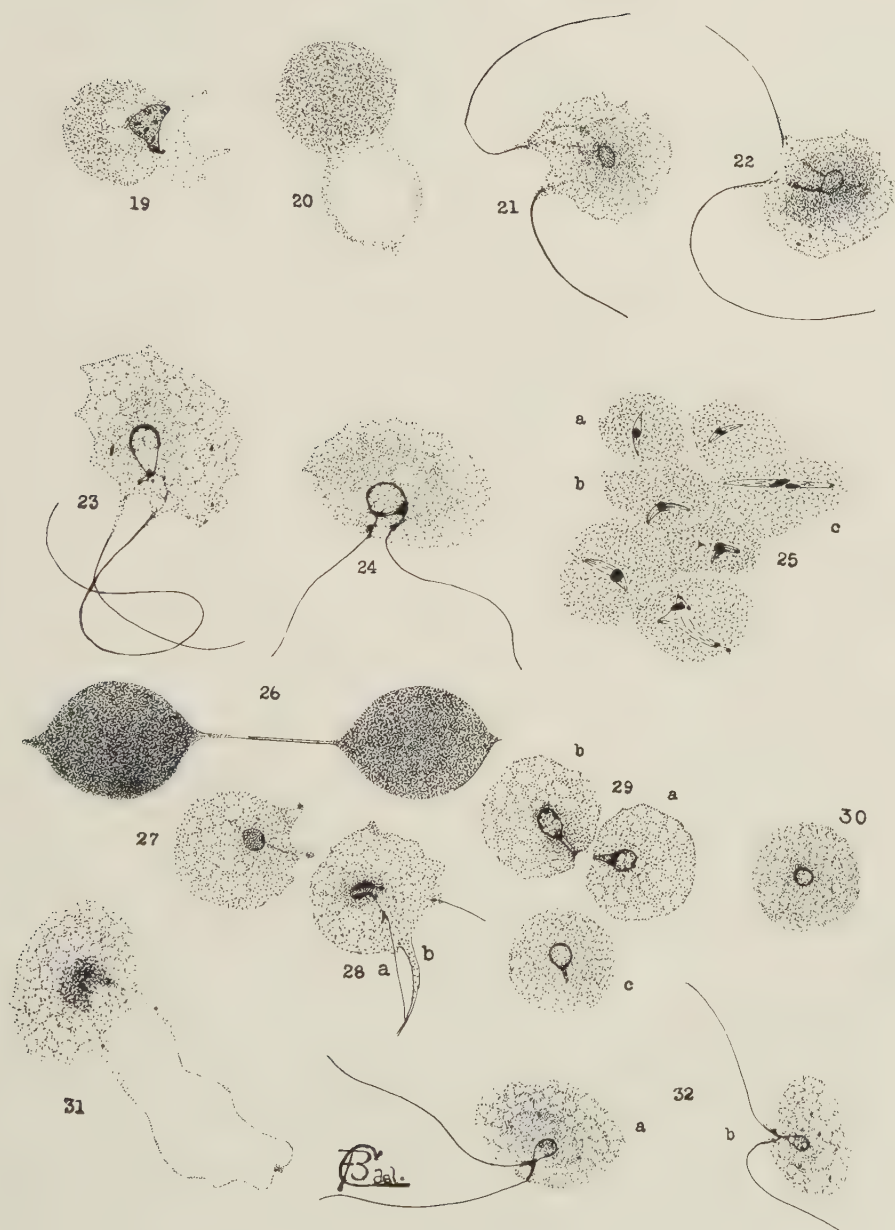
FIG. 42. An active spore showing separate basal granules with connecting strands inserted in the same dense chromatic body in the nucleus. The cytoplasm is finely granular and more dense toward the center and more vacuolate toward the periphery.

FIG. 43. Spore with three cilia, two of which are apparently separate from the nucleus, evidently the result of one basal apparatus becoming completely separated from the nucleus.

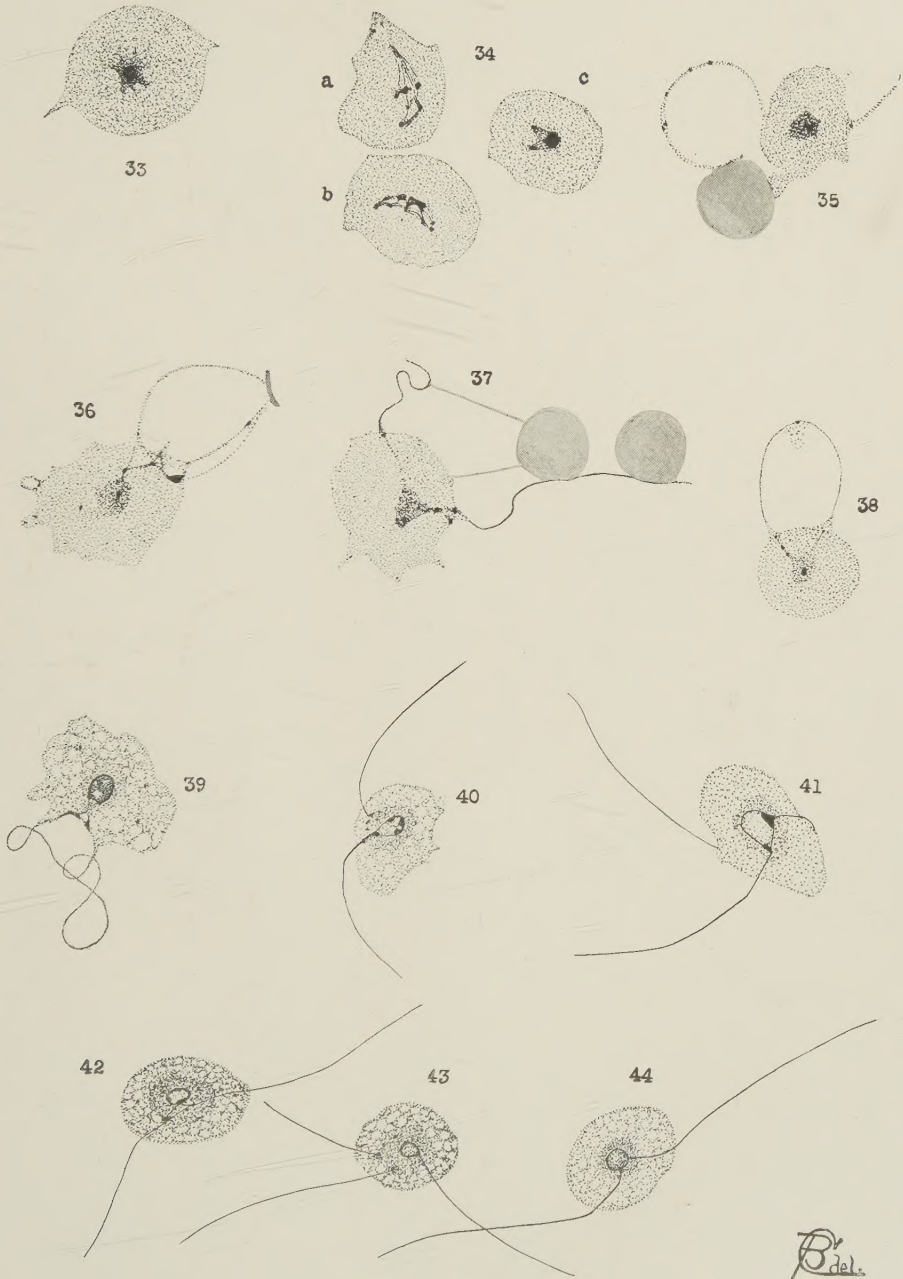
FIG. 44. Spore in which the cilia seem to be attached directly with the nucleus; the basal granule is evidently near the surface of the nucleus.



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